Perceptual and Motor Skills, 1998 86. 1288 1290. © Perceptual and Motor Skills 1998

"SUBCLINICAL" DOSAGES OF LITHIUM AND PILOCARPINE THAT DO NOT EVOKE OVERT SEIZURES AFFECT LONG-TERM SPATIAL MEMORY BUT NOT LEARNING IN RATS[1]

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Summary.—After training in an automated radial maze, 11 male rats were injected with either "subclinical" dosages of lithium and pilocarpine or saline and then tested 5 days or 4 months later. When employed as their own controls or when compared with a saline-injected reference group, the rats that had received the lithium and pilocarpine displayed memory deficits but not learning deficits after the longest of the two delays (effect size was 41%). These results suggest that subtle disruptions in memory but not learning to criterion could be associated with "subclinical electrical seizures" or the micromorphological changes associated with this activity.

Within humans and rats the occurrence of subclinical limbic seizures subsequent to traumatic (acquired) brain injuries have been considered benign. Reports of alterations in memory by patients with complex partial epileptic-like signs have been considered misattributions or secondary to psychiatric or "psychological" factors (Persinger, 1995). One experimental model of limbic seizures in the rat can be induced by a single systemic injection of lithium (3 mEq/kg) and pilocarpine (30 mg/kg). For the last ten years we have found that, if rats do not display overt seizures in response to this treatment, they do not display (400 ×) microscopic brain injury (Persinger, Bureau, & Peredery, 1994).

Although Lewis rats that had been inoculated (footpads) with an emulsion of spinal cord and Freund's complete adjuvant at the time they were injected with "subclinical" dosages (1.5 mEq/kg) of lithium and pilocarpine (15 mg/kg) did not exhibit 15 days later the discernable neuronal damage that accompanies overt limbic seizures (3 mEq/kg of lithium; 30 mg/kg of pilocarpine), there were conspicuous infiltrations of mononuclear cells (lymphocytes) within white matter such as the fornix, fimbria and alveus of the hippocampal formation (Cook & Persinger, in press). This infiltration, which suggests an alteration in the blood-brain barrier without neuronal necrosis, was not observed in age-matched Lewis rats that had not received the subclinical dosage of lithium and pilocarpine. Considering the role of these structures in visuospatial memory, the present experiment was designed to discern the potentially subtle effects of this concentration of lithium and pilocarpine upon the memory and the learning of a radial maze.

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A total of 11 male, 120-day-old Wistar rats were maintained at 85% body weight. They were tested within an automated four-arm radial maze for two days (Persinger, *et al.*, 1994). The time to complete the first successful trial defined by pressing the operant lever (for a food pellet) at the end of each arm, the numbers of trials per session, the numbers of bar presses per reward, and the average time to complete each trial were recorded for two days.

Two weeks later, six of the rats were injected subcutaneously with 1.5 mEq/kg of lithium chloride and then 4 hr. later with 15 mg/kg of pilocarpine. The five controls received injections of isotonic saline. There were no overt seizures or unusual behaviours during the hour after the lithium and pilocarpine injections and no changes in body weights during the subsequent week. Each rat was tested for two days in the maze five days and 4 mo. later. The six measurements (2 days × 3 times) for each of the four measures were standardized for each rat (z scores) so the individual variations would be minimized. Three-way analysis of variance with two factors repeated (time of testing and days) and one not repeated (saline vs lithium and pilocarpine) for each of the four measures and *post hoc* comparisons were completed by SPSS software. The means and standard deviations for these z scores are presented in Table 1.

TABLE 1

MEANS AND STANDARD DEVIATIONS FOR WITHIN-SUBJECT z SCORES FOR FOUR MEASURES OF MAZE PERFORMANCE BY RATS INJECTED WITH SALINE (CONTROL GROUP) OR SUBCLINICAL DOSAGES OF LITHIUM AND PILOCARPINE (EXPERIMENTAL GROUP)

Measure	Preinjection				Posttest 5 Days				Posttest 4 Months			
	Day 1		Day 2		Day 1		Day 2		Day 1		Day 2	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Time to Complete First Trial												
Controls	−.5	.3	−.1	.9	−.7	.3	.6	.9	.4	1.1	.3	1.0
Experimental	−.1	.7	−.4	.4	−.7	.6	−.3	.6	1.6	.4	−.1	.7
Total Numbers of Completions												
Controls	.4	.5	.7	.5	.7	.6	.2	.8	−1.3	.3	−.7	.6
Experimental	−.2	.3	.9	.7	.6	.4	.5	.9	−1.1	.4	−.7	.4
Average Numbers of Bar Presses												
Controls	−.3	.7	−.6	.3	−.6	.4	.3	1.1	1.0	.7	.2	1.1
Experimental	.3	.7	−.7	.7	−.7	.5	.0	.9	.5	.8	.7	1.0
Average Time to Complete Each Trial												
Controls	−.5	.4	−.5	.3	−.7	.3	−.4	.6	1.5	.5	.7	.8
Experimental	.0	.6	−.8	.5	−.6	.3	−.5	.8	1.2	.7	.7	.6

There were no statistically significant mean differences between the two treatments. The only statistically significant interaction between the treatments and either time (5 days and 4 months) or days occurred with the la-

tency for completion of the first trial. For this measure there was a statistically significant ($F_{1,9}=7.94$, $p<.05$; $\eta^2=47\%$) interaction between treatment and the first vs the second day of testing. Additional analysis, however, indicated that the major contribution of this effect was due to the longer time ($F_{1,9}=6.74$, $p<.05$; $\omega^2=41\%$) required for the treated rats ($Mz=1.6$, $SD=0.4$) to complete the first trial relative to the saline-injected group ($Mz=0.4$, $SD=1.1$) on the first day only when tested at 4 mo. All four measures, latency to complete the first trial, total numbers of trials, average time to complete each trial, and numbers of bar presses per reinforcement, displayed the expected effect ($F_{1,9}>7.00$, $p<.001$) for time of testing (baseline, 5 days, and 4 mo.) and reflected the discrepancies for scores between testing at 4 mo. and during either baseline or 5 days after the injections.

These results suggest that, when each rat is employed as its own control, subtle changes in spatial memory can be discerned following injections of substances considered ineffective in evoking overt, conspicuous behaviors. The specific deficits for the first day of testing 4 mo. after the treatment may be inferred as a deficit in long-term memory but not in the capacity to relearn. This would be consistent with interference from subtle limbic electrical activity or slight disruption of the blood brain barrier within the white matter of the hippocampal system. This may remain recondite within both experimental and clinical populations.

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Accepted May 6, 1998.